AMENDMENTS

IN THE SPECIFICATION:

Please insert the enclosed paper copy of the Sequence Listing after the abstract on page 177 as separately numbered pages 1-9.

At page 21, line 22 after "Brief Description of the Drawings", insert the following paragraph:

This patent application as filed contains at least one drawing executed in color. Copies of this patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Amend the paragraph beginning at page 21, line 23 as follows:

~~Figure 1 show~~ **Figures 1A and 1B show** the amino acid sequences of rat SIK1 **protein, human SIK2 protein, and** ~~and~~ mouse SIK2 **protein** ~~proteins~~ **(SEQ ID NO:2)**;

Amend the paragraph beginning at page 124, lines 26 as follows:

~~Figure 7(a)~~ **Panel A of Figure 7** shows the results indicating the reaction between a wild-type peptide or a peptide wherein serine at position 577 is substituted with alanine, and an antibody. The term "CBB" represents Coomassie brilliant blue staining. CBB is used as a control substance showing that there is no reactivity when it does not exist and that there is reactivity when it exits. As shown in **Panel A of Figure 7** ~~Figure 7(a)~~, it was found that GST of a carrier protein did not react, that a wild-type peptide that was phosphorylated by PKA reacted, and that a peptide that became unphosphorylated by substituting serine at position 577 with alanine did not react. The lower experiment in **Panel A of Figure 7** ~~Figure 7(a)~~ shows that when a wild-type peptide is not phosphorylated by PKA, it does not react.

Amend the paragraph beginning at page 125, line 22 as follows:

As is clear from **Panel B of Figure 7** ~~Figure 7(b)~~, it was confirmed that the present antibody is capable of detecting the phosphorylation state of domain 3.

Amend the paragraph at page 126, line 15 as follows:

As shown in **Panel A of Figure 8** ~~Figure 8(a)~~, it is found that a peptide wherein serine at position 89 in p300 is substituted with alanine becomes unphosphorylated.

Amend the paragraph beginning at page 126, line 18 as follows:

As shown in **Panel B of Figure 8** ~~Figure 8(b)~~, it is found that the unphosphorylated peptide p300 wherein serine at position 89 is substituted with alanine inhibits the enzyme activity of SIK. The left lane and the central lane show the results regarding a synthetic substrate (Syntide 2) phosphorylated by SIK.

Amend the paragraph beginning at page 126, line 24 as follows:

Panel C of Figure 8 ~~Figure 8(c)~~ shows the experiment results obtained by examining the effects of a peptide having inhibitory activity on CRE activity. GFPC is a C-terminus binding-type green fluorescent protein. GFPC is used as a peptide carrier, wherein a peptide of interest binds to the C-terminus of GFP. The left shows a control, and the right shows the CRE activity obtained when p300 [84-93 (S89A)] binds thereto. The black one represents CRE activity obtained in a case where only PKA exists but SIK does not exist. The grey one is CRE activity obtained when both PKA and SIK exist. Thus, it was shown that p300 [84-93 (S89A)] inhibits the CRE-suppressing activity of SIK in cells, that is, the phosphorylating activity thereof.

Amend the paragraph beginning at page 127, line 14 as follows:

To the CRE assay system in the precursor fat cells in Example 5, 50 ng/ml Tumor Necrosis Factor-α (TNFα) was added. Thereafter, the same experiment as in Example 5 was carried out. It is to be noted that it has been reported that TNFα inhibits CREB. The results are shown in Figure 9. As is clear from **Panel A of Figure 9** ~~Figure 9A~~, even

when SIK2 was not highly expressed, TNFα sufficiently suppressed the activation of CRE due to PKA.

Amend the paragraph beginning at page 127, line 22 as follows:

In addition, CREB was introduced into the N-terminal side of the Gal4 protein of budding yeast (Gal4 DNA binding domain; Gal4DB). Using a plasmid wherein a reporter gene (fire fly luciferase) was introduced downstream of a promoter having a sequence consisting of 5 consecutive Gal4-binding sequences, the ability of DB to transcribe CREB without the medium of CRE was analyzed. In the case of a Gal-CREB (full-length) system, since it has a bZIP domain, the transcription was inhibited by SIK2 and TNFα (refer to **Panel B of Figure 9** ~~Figure 9B~~).

Amend the paragraph beginning at page 128, line 6 as follows:

In addition, in the case of a Gal4-CREB(-bZIP) system, it was found that since there were no working points (bZIP) of SIK2, the transcription was not suppressed by SIK2, but that the transcription was suppressed by TNFα (refer to **Panel C of Figure 9** ~~Figure 9C~~).

Amend the paragraph beginning at page 131, line 25 as follows:

The results are shown in Figure 11. Figure 11 shows the results obtained by assaying the mRNA level of various types of fat cell differentiation markers, which is induced in the differentiation process of fat cells. As shown in **Panel A of Figure 11** ~~Figure 11(a)~~, after completion of the initial culture for 24 hours, mRNA of SIK2 was significantly expressed. The mRNA level was maintained high until almost all precursor fat cells were differentiated into mature fat cells after 7 days (judged by oil Rel-O staining). On the other hand, the expression level of mRNA of SIK1 was much lower than that of SIK2. (In **Panel A of Figure 11** ~~Figure 11(a)~~, the exposure time of the film was different in SIK1 and SIK2.) However, the expression level increased for the initial 24 hours, and the concentration was maintained until the 7th day. The mRNA level of Pref-1 as a marker of precursor fat cells began to decrease on the 2nd day. The mRNA levels of c/EBPβ and c/EBPδ that were known transcriptional factors appearing at the

initial stage of fat differentiation increased for the initial 24 hours, as in the case of SIK2. SREBP-1, c/EBPα, PPARγ, and aP2 are known as late responsive genes in the generation of fats. The mRNA level of these genes began to increase on the 2nd or 4th day.

Amend the paragraph beginning at page 132, line 22 as follows:

With regard to c/EBPβ and c/EBPδ that are known to appear at the initial stage of fat differentiation, dibutyl cAMP (1 mM) was used as a constituent for differentiation cocktail mix, instead of methylisobutylxanthin for the differentiation cocktail mix. The expression level of mRNA was examined until 1 to 12 hours after stimulation for differentiation. The results are shown in **Panel B of Figure 11** ~~Figure 11(b)~~. As shown in **Panel B of Figure 11** ~~Figure 11(b)~~, the mRNA levels of both c/EBPβ and c/EBPδ increased within 1 hour after the stimulation. Two hours later, however, the mRNA level of c/EBPδ gradually decreased.

Amend the paragraph beginning at page 133, line 7 as follows:

It was examined which one of the 3 types of hormones, insulin, cAMP, and dexamethasone (DX) contained in a mixture thereof, stimulates the transcription of an SIK2 gene. The SIK2 gene was incubated with each of these hormones, and with the mixture consisting of the 3 types of hormones, for 2 hours. Thereafter, the mRNA level of SIK2 was measured. With regard to the concentration of hormones used, insulin was 1 μg/ml, cAMP was 1 mM, and dexamethasone was 1 μM. The measurement was carried out by the Northern blot analysis using cDNA probes. The results are shown in **Panel C of Figure 11** ~~Figure 11(c)~~. As shown in **Panel C of Figure 11** ~~Figure 11(c)~~, insulin and cAMP promoted the transcription of the SIK2 gene when they were used singly. When dexamethasone was used singly, it activated the SIK2 gene at the level equivalent to that obtained by the mixture consisting of the 3 types of hormones.

Amend the paragraph beginning at page 136, line 4 as follows:

The results are shown in **Panel A of Figure 13** ~~Figure 13(a)~~. In the figure, WT represents wild-type mice and db represents db/db/ fat diabetic mice. In addition, WAT represents white adipose cell tissues, BAT represents brown adipose cell tissues, Liver

represents the liver, and SkM represents the skeletal muscle. As shown in **Panel A of Figure 13** ~~Figure 13(a)~~, in both cases of the wild-type mice and the db/db/ fat diabetic mice, SIK2 mRNA was detected at the same level in the white adipose cell tissues and in the brown adipose cell tissues. In contrast, in both cases of the wild-type mice and the db/db/ fat diabetic mice, the concentration of SIK2 mRNA was extremely low in the liver and the skeletal muscle. The level of mRNA was the same in the wild-type mice and the db/db fat diabetic mice. As a control, the level of SIK1 mRNA was detected. The level of SIK1 mRNA in the brown adipose cell tissues, the liver, and the skeletal muscle was greater in the db/db fat diabetic mice than in the wild-type mice.

Amend the paragraph beginning at page 136, line 24 as follows:

A protein was purified by the immunoprecipitation method from a homogenate containing 3 mg each of the white adipose cell tissues and the brown adipose cell tissues, and 18 mg each of the liver and the skeletal muscle obtained in Example 14. SIK2 was then detected by the Western blot analysis. The Western blot analysis was carried out using an anti-SIK2 antibody after SDS-PAGE. The results are shown in Figure 13(b). As shown in **Panel B of Figure 13** ~~Figure 13(b)~~, it is found that the amount of the SIK2 protein in the white adipose cell tissue of the db/db fat diabetic mice was larger than that of the wild-type mice. On the other hand, in the case of the brown adipose cell tissues, the amount of the protein in the db/db fat diabetic mice was smaller than that in the wild-type mice. In the liver and the skeletal muscle, the amount of SIK2 was small, and thus, almost no difference was observed between the db/db fat diabetic mice and the wild-type mice

Amend the paragraph beginning a page 137, line 17 as follows:

Subsequently, with regard to SIK2 purified by immunoprecipitation from the tissues used in Examples 14 and 15, the kinase activity *in vitro* was assayed using GST-Syntide 2 as a substrate. The results are shown in **Panel C of Figure 13** ~~Figure 13(c)~~. As shown in **Panel C of Figure 13** ~~Figure 13(c)~~, it is found that the SIK2 activity in the white adipose cell tissue of the db/db fat diabetic mice was higher than that of the wild-type mice. On the other hand, in the case of the brown adipose cell tissues, although the

SIK2 activity was low both in the wild-type mice and in the db/db fat diabetic mice, the activity of the wild-type mice was higher than that of the db/db fat diabetic mice. As is clear from the results shown in **Panel B of Figure 13** ~~Figure 13(b)~~, it is suggested that the obtained results correspond to a difference in the content of the SIK2 protein. The liver homogenate had SIK2 activity. The activity in the liver homogenate in the db/db/ fat diabetic mice was slightly higher than that in the wild-type mice. The skeletal muscle homogenate did not exhibit SIK2 activity that was detectable. These results show that the amount of the SIK2 protein increased in the white adipose cell tissues of the diabetic mice, as in the case of the activity thereof.

Amend the paragraph beginning at page 139, line 24 as follows:

The results are shown in **Panel A of Figure 14** ~~Figure 14A~~. As shown in **Panel A of Figure 14** Figure 14A, the wild-type SIK1 was phosphorylated, but the mutants S577A, R574A, and R574/575A were not phosphorylated. On the other hand, the mutant R575A was phosphorylated much more efficiently than the case of the wild-type SIK1. The reaction in the present example is an intermolecular reaction using an SIK peptide as a substrate. Since serine at position 577 of SIK1 exists in a molecule of SIK1, the concentration of the substrate is much higher than that in a test tube. Thus, it is predicted that serine at position 577 is efficiently phosphorylated by SIK1 domain 1.

Amend the paragraph beginning at page 140, line 12 as follows:

Subsequently, using phosphorylated GST-SIK1 and phosphorylated GST-SIK2 as substrates, and using the antibody obtained in Example 8, the Western blot analysis was carried out. The results are shown in **Panel B of Figure 14** ~~Figure 14B~~. As shown in **Panel B of Figure 14** ~~Figure 14B~~, it was found that the antibody obtained in Example 8 recognizes the auto-phosphorylation states of serine at position 577 of SIK1 and serine at position 587 of SIK2. It is to be noted that the sequence adjacent to position 577 of SIK1 is conserved in a mouse, a rat, and a human.